DISCUSSION

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MC and MD have significantly decreased the serum glucose level in streptozotocin induced diabetic rats. Treating the FRD fed rats with MC (250 & 500 mg/kg p.o/day/15days), the serum glucose level significantly decreased. Treatment with MC (250 & 500 mg/kg p.o/day) or MD (250 & 500 mg/kg p.o/day) to AD rats also significantly decreased the serum glucose level. Momordica charantia (Sugihara Y et al 2005), Gymnema sylvestre (Sugihara Y et al 2000), Ocimum sanctum(Gupta S et al 2006) etc plants have also shown antihyperglycemic activity in various metabolic disorders like streptozotocin induced diabetes, FRD induced insulin resistance, HFD induced hyperlipidemic rats.

The mechanisms of both allopathic medicines and the traditional herbal medicines to lower blood glucose are (W.L. Li et al., 2004):

- to stimulate beta -cell of pancreatic islet to release insulin;
- to resist the hormones which rise blood glucose;
- to increase the sensitivity of insulin receptor site to insulin;
- to decrease the leading-out of glycogen;
- to enhance the use of glucose in the tissue and organ;
- to clear away free radicals, resist lipid peroxidation and
- correct the metabolic disorder of lipid and protein;

Streptozotocin is well known for its selective pancreatic islet of β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio G et al., 2000). Intraperitoneal administration of streptozotocin (65 mg/kg) effectively induced diabetes in normal rats. MC increased serum insulin level significantly in overnight-fasted diabetic rats. This observation indicates that MC enhances insulin release from destroyed pancreatic β-cells, either by regenerating the partially destroyed pancreatic beta cells or by the release of insulin stored in the granules. In order to know whether the increase in serum insulin level after administration MC was due to the release of insulin stored in the granules or due to direct effect on the pancreatic islets of β-cells, histopathology of the pancreas was studied. Histopathological sections of the pancreatic islets of diabetic rats showed irregularly shaped, small and scanty islets with severe vacuolation and degranulation in the β-cells of a number of islets.

Histopathological sections of the pancreatic islets in MC and its saponins treated rats showed increase in the number of pancreatic cell islets which were similar to that of healthy pancreatic islets and reversed the atrophy of the pancreatic islets of β-cells. The regeneration of the β-cells of the STZ-destructed islets is probably due to the fact that pancreas contains stable (Quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells (Kumar V et al 1992; Govan, A.T et al., 1986). *Gynmnema Sylvestre* also increases insulin secretion probably by regeneration of pancreatic beta cells (Baskaran K et al., 1990, Shanmugasundaram ER et al 1990). In vitro trials on experimental models with *Gynmnema Sylvestre* have proved that it increases insulin release by increasing the cell permeability (Persaud SJ et al 1999).Many other plants like *Teucrium polium*(Razieh Yazdanparast et al., 2005), *Aegle marmelos* (N. Kamalakkannan et al ., 2006) *Ephedra sinica Stapf., and Ephedra distachya L*(Xiu et al.,2001), *Prunella vulgaris L.*(Liu B.L et al., 1995) are reported to regenerate atrophied pancreatic islets, restore the secretion of insulin, and thus correct hyperglycemia. *Momordica Charantia* fruit is reported to have insulin secretagogue and insulinomimetic activity (Raman and Lau, 1996). *Trigonella foenum-graecum* (Sauvaire et al., 1998) and *Allium sativum* L (Augusti and Sheela, 1996) are reported to act by stimulating insulin secretion. One of the main constituent of Momordica charantia is a steroidal saponin-charantin, and is responsible for the fruit's anti-diabetic effects; it also contains Momordicine and insulin-like steroidal saponin. They also have a group of triterpenes including momordicosides, A-E, K, L, and momardicius I, II and III. (M.F. Mahomoodally et al., 2007).

Saponins isolated from plants such as fenugreek (Petit et al.1993), Phellodendron cortex and Aralia cortex (Kim et al.1998c), Pueraria thunbergiana (Lee et al. 2000a), and Calendula officinalis (Yoshikawa et al. 2001) have been shown to cause hypoglycaemic effects. Petit et al. (1993) found chronically higher plasma insulin levels, probably caused by stimulation of the β-cells in male Wistar rats. The saponin momordin Ic was also found to significantly and dose-dependently inhibit gastric emptying (Matsuda et al. 1999a).

Saponin glycoside, christinin-A, B, C and D were isolated from *Zizyphus spina-christi* (L.), (Ahmed O et al 2005).

D-glucose induces a rise in pancreatic islet beta-cell cytosolic [Ca2+] by processes requiring both glucose metabolism and Ca2+ entry from the extracellular space, and

this Ca2+ signal is thought to be critical to the induction of insulin secretion. Insulin secretagogues also induce phospholipid hydrolysis and accumulation of phospholipid-derived mediators in islets, including the lipid messengers DAG, nonesterified arachidonic acid, and arachidonate 12-LO products. Hepatic glycogen is decreased in diabetes mellitus and this is the most important cause of diabetes ketosis and coma because of impoverishment of liver with respect to glycogen. Increase in glycogen in liver can be brought about by an increase in glycogenesis and/or a decrease in glycogenolysis. Insulin acts on liver cells by stimulating them to take up glucose from the blood and convert it into glycogen and inhibiting glycogenolysis and gluconeogenesis. In the present study a significant decrease in hepatic glycogen level was seen in the streptozotocin induced diabetic rats. Treatment with MC significantly increased the hepatic glycogen level.The increased glycogen level may be due increased level of insulin in the MC & MD treated diabetic rats which has increased glycogenesis and decreased glycogenolysis and gluconeogenesis. The anti-hyperglycemic mechanism of *Allium sativum* L.' is to stimulate in vitro insulin secretion from β-cells of pancreatic islet, increase serum insulin level, and improve glucose tolerance and increase liver glycogen synthesis (Sheela and Augusti, 1996).

Insulin resistance is a common phenomenon of the patient with type 2-diabetes and it usually antedates the onset of the disease. Insulin resistance prevents the target tissues (especially the muscle and liver) from responding to normal circulating concentrations of insulin. It affects glucose disposal in muscle and fat and reduces insulin suppression of hepatic glucose output. Insulin deficiency develops over time—initially as a loss of first-phase insulin release with a compensatory increase in the second phase.

This hyperinsulinemic response does not persist and the insulin secretory capacity of the beta cell begins to wane. High concentration of fructose can result in a relatively unregulated concentration of dihydorxy acetone phosphate (DHAP) and d-glyceraldehyde (Hallfrisch J 1990). The induced production of DHAP and D-glyceraldehyde individually results in uncontrolled synthesis of glucose, acetyl CoA, triglyceride and fatty acids (Verschoor L et al 1985).This stimulated triglyceride synthesis is likely to lead to hepatic accumulation of glycerol, fatty acids and triglycerides that have been shown to reduce insulin sensitivity (Poitout V 2002).Increased fructose may also alter insulin sensitivity partially by altering insulin binding. Thus fructose rich diet results in a state of hyperglycemia,

hypertrigylceridemia and increased free fatty acid levels. Hyperglycemia has been implicated in the activation of additional biochemical pathways, which are linked to the development of IR and ß-cell dysfunction (Joseph LE et al., 2002).The present study also indicated that fructose-induced hyperglycemia, is associated with significant hyperinsulinemia hypertrigylceridemia and hypercholesterolemia. MC and MD significantly attenuated hyperinsulinemia in FRD rats. Hence MC and MD may act as insulin sensitizers.

Brassica juncea (Rai) significantly prevented the development of insulin resistance in rats fed fructose-enriched diet (Yadav et al., 2005). Oral administration of the aqueous extract from *A. senticosus* root had the ability to improve insulin sensitivity and delay the development of insulin resistance in rats (Tsang-Pai Liu et al., 2005) Treatment with extracts of *Momordica charantia* and *Eugenia jambolana* prevented hyperglycemia and hyperinsulinemia in fructose fed rats(Vats Vikrantet al.,2001).

The 3 major components of the dyslipidemia of insulin resistance are increased triglyceride levels, decreased high-density lipoprotein (HDL) cholesterol, and changes in the composition of low-density lipoprotein (LDL) cholesterol. Hyperinsulinemia and the central obesity that typically accompanies insulin resistance are thought to lead to overproduction of very low-density lipoprotein (VLDL) cholesterol. The result is more triglyceride-rich particles, fewer HDL particles, and smaller, dense LDL. Postprandial triglyceride levels and measures of postprandial remnants also may contribute to increased coronary artery disease (CAD) risk in individuals with insulin resistance. (Howard BV et al., 1999) Insulin resistance and hyperinsulinemia are also associated with an atherogenic plasma lipid profile. Elevated plasma insulin concentrations enhance very-low-density lipoprotein (VLDL) synthesis, leading to hypertriglyceridemia. Progressive elimination of lipid and apolipoproteins from the VLDL particle leads to an increased formation of intermediate-density and low-density lipoproteins, both of which are atherogenic.

Deficiency in insulin in diabetes leads to elevated levels of free fatty acids in the plasma as a result of uncontrolled lipolysis in adipose tissue. In uncontrolled IDDM there is a rapid mobilization of triglycerides leading to increased levels of plasma free fatty acids. The free fatty acids are taken up by numerous tissues (however, not the brain) and metabolized to provide energy. Free fatty acids are also taken up by the liver. In the absence of insulin, malonyl-CoA levels fall and transport

of fatty acyl-CoA's into the mitochondria increases. Mitochondrial oxidation of fatty acids generates acetyl-CoA which can be further oxidized in the TCA cycle. Plasma triglycerides are also acted upon by lipoprotein lipase (LPL), activity of LPL requires insulin and in its absence a hypertriglyceridemia results.

Diabetic rats treated with MC (250 & 500mg/kg, p.o/day/30days) and MD (250 & 500mg/kg, p.o/day/30days) significantly decreased the serum cholesterol and triglyceride level. Since insulin is a major harmone regulating lipid metabolism, MC/MD fecilitated stimulation of insulin secretion in STZ rats will help overcome lipid metabolism abnormalities.

Type I diabetes is associated with lower rates of cholesterol synthesis and increased absorption of dietary cholesterol (Miettinen T.A et al 2004). These individuals are at high risk for the development of cardiovascular disease (Armitage J & Bowman L 2004), and have higher total serum cholesterol levels. In rats, streptozotocin-induced diabetes also renders animals particularly susceptible to a dietary cholesterol insult (Ness G.C.& Gertz K.R 2004). For reasons that are still unclear, this sensitivity correlates well with decreased expression of hepatic HMG-CoA reductase (Ness G.C. & Gertz K.R 2004b), the enzyme that catalyzes the rate-limiting reaction in cholesterol biosynthesis. Hepatic HMG-CoA reductase is responsible for the majority of the body's regulatable cholesterol synthesis. The expression of this enzyme is affected by cholesterol, insulin, thyroid hormone, bile acids, fasting and re feeding, and also varies diurnally (Ness G.C.& Chambers C.M. 2000). HMG-CoA reductase (HMGR) protein and mRNA levels are both decreased in diabetic animals, and can be rapidly restored with insulin treatment (Ness G.C et al., 1994).

Saponin fractions of MC showed a significant inhibition of HMG CoA reductase activity. Saponin fractions of MC has also showed a significant inhibition of HMG CoA reductase activity in HCD fed rabbits.Hence the antihypercholesteremic effect seen may be due to increased secretion of insulin in diabetic animals and HMG CoA reductase inhibitory activity of MC.

Creatinine, the end product of creatine metabolism, forms continuously at a relatively constant rate based on skeletal muscle mass. Normally, all creatinine produced by the body is filtered continuously and excreted by the kidneys, so creatinine levels relate directly to the glomerular filtration rate and are highly specific indicators of impaired renal function. Billstrom A. (1989) has observed a significantly

higher increase in plasma creatinine among the patients with diabetic nephropathy compared with those without nephropathy. An increased albumin excretion rate (AER) is associated with impaired glucose tolerance and diabetes mellitus. Urinary creatinine concentration (UCC) is widely used to estimate renal involvement in diabetes.

Streptozotocin (65mg/kg i.p./single dose) induced diabetic rats in our study showed a significant increase in serum creatinine level. Diabetic rats treated with MC (250&500mg/kg, p.o/day/30days) significantly decreased serum creatinine level. Diabetic rats treated with MD (250&500mg/kg, p.o/day/30days) also showed a significant decrease in the creatinine level.

Urea, the major end product of protein metabolism, forms in the liver from ammonia and is excreted via the kidneys. Blood urea nitrogen measures this nonprotein nitrogenous waste and reflects both protein intake and renal excretory function. Serum Blood Urea Nitrogen (BUN) level showed a significant increase in streptozotocin induced diabetic rats. Treatment with MC (250 & 500mg/kg, p.o/day/30days) significantly decreased BUN level in diabetic rats.

A.M. Carpenter (1993) has reported that Type 2 diabetes in later life appears to be associated with a high risk for typical tissue changes of diabetic kidney damage, which may contribute significantly to morbidity and mortality and may be present before azotaemia and qualitative proteinuria have been recognized. Histological section of diabetic kidney shows 1) typical diabetic nodular glomerulosclerosis; 2) mesangial changes suggestive of diabetes (diffuse lesion); 3) non-diabetic renal disease.

Histopathological sections of the diabetic rats in our study showed severe glomerular sclerosis, arteriolar hyalinization, cortical interstitial fibrosis and increased number of vacuoles which may be due to glycogen deposition (pas positive) Histopathological sections of the MC (500mg/kg p.o./day/30days) treated rats showed a minimal glomerular sclerosis and vacuolization.

Hence MC and MD give protection against diabetic nephropathy. The effect may be due to insulin secreting or insulin sensitizing mechanism of MC& MD. Insulin sensitizer, metformin, is also reported to protect against gentamycin-induced nephrotoxicity in rats. Momordica charantia protects from nephrotoxicity in diabetes (Kumar Shetty A et al., 2005).

Diabetes remains a major risk factor for heart failure and death complicating acute MI. Myocardial histology in diabetic heart failure, that includes myocyte hypertrophy, interstitial fibrosis, increased PAS-positive material and intramyocardial microangiopathy (Sreekumar Sulfi 2006) is similar to changes found in hypertensive left ventricular disease. Thus, the specific heart muscle disease that characterizes diabetes may contribute importantly to the heightened risk of heart failure by diastolic and systolic mechanisms.

Patients with diabetes due to an absolute or relative deficiency of insulin may show acute myocardial infarction as a result of high levels of plasma FFA. The high levels of circulating and cellular FFAs inhibit oxidative glucose metabolism and result in local depletion of ATP and accumulation of toxic metabolic intermediates. This has the potential to worsen ischaemia, leading to extension of infarction and inhibition of myocardial contractility remote from the infarct zone. Therapeutic strategies which increase local delivery of glucose and inhibit FFA utilisation may have a favourable influence.

Histopathological sections of the heart of diabetic rats showed infiltration of the inflammatory cells without continuity in muscle fibers suggesting an irreversible cell injury. Histopathological sections of the MC (500mg/kg p.o/day/30days) treated rats showed normal regenerative changes with striations, branched appearance and continuity with the adjacent myofibrils. The cardioprotective effect of MC in diabetic rat may be because it inhibits FFA utilization due to insulin releasing effect of MC.

Atherogenesis is directly mediated not by lipids, but by lipoprotein particles, both "bad" (LDL, VLDL) and "good" (HDL): "...all abnormalities in plasma lipid concentrations, or, can be translated into dyslipoproteinemia". Studies have demonstrated that oxidized LDL plays important role in the initiation and progression of atherosclerosis (Steinberg D et al.,1989).Minimally modified LDL is capable of inducing gene expression in the endothelial cells that may result in the acceleration of atherosclerosis(Cushing SD 1990).On further modification of the intima oxLDL is further taken up by the macrophage scavenger receptor gradually leading to the formation of foam cells and fibrous plaques (Partthasarathy et al., 1986).Drugs that prevent LDL from oxidative damage may interrupt the progression of atherosclerosis.

Treatment with MC or MD and their saponins in various metabolic disorders like STZ induced diabetic rats, CCl_4 induced hepatoxic rats significantly reduced lipid peroxidation or MDA content in the heart as compared to STZ induced diabetic or

CCl_4 control group and prevents LDL from oxidative damage and interrupt the progression of atherosclerosis.

Prospective data from the Quebec Cardiovascular Study (Benoit Lamarche et al., 1997) indicate that elevated LDL particle numbers and small LDL size predict CHD risk better than LDL cholesterol. Growing evidence implicates the number of LDL particles as the key risk factor. Lipoprotein movement is a gradient driven process and an excessive number of LDL particles set the stage for large amounts of cholesterol to enter the sub endothelial space. Particle size modulates the level of risk only when excessive numbers are present. Therefore quantifying the number of LDL particles is essential to assess risk. The Quebec Cardiovascular Study also found that LDL particle size plays a role in determining the degree of risk only when there are an elevated number of LDL particles.

Data from the Framingham Offspring Study shows that over 50% of all individuals that go on to develop heart disease have "normal" cholesterol values. Normocholesterolemic individuals with a higher concentration of the small, dense LDL particle are at increased risk for coronary heart disease (Austin MA et al., 1988, Tornvall P et al., 1991, Campos H et al., 1992). Numerous studies indicate that particle subclasses of LDL, HDL, and VLDL have differing associations with CHD. Small, dense LDL is more atherogenic than large LDL, large HDL subclasses are more protective than small HDL, and very large VLDL is more strongly associated with CHD than smaller VLDL. Because of underlying differences in lipoprotein subclass distribution and metabolic status, patients with the same lipid panel values can have substantially different risks of CHD. Treatment with MC or MD to AD rats or diabetic rats or HFD rats or HCD rabbits significantly reduced LDL level compared to respective in control animals, and hence MC or MD may show a significant protection in dyslipidemia

The complications from atherogenic dyslipidemia stem from alterations in lipoprotein metabolism and lipoprotein particle morphology. Abnormalities in VLDL particle size are considered a major contributing factor to dysfunctional lipoprotein metabolism (Millar JS & Packard CJ 1998). Elevated hepatic TG levels lead to the secretion of large VLDL particles, which are most susceptible to modification by hepatic lipase (HL). Through the delipidation cascade, TG-rich VLDL is the precursors for the formation of small, dense LDL particles (Zambon A et al., 2003). The phenotype characterized by a predominance of small LDL particles is termed "pattern B" and is

characteristic of the metabolic syndrome (Austin MA et al 1990, Packard CJ 2003). Small, dense LDL particles are considered more atherogenic because of a decreased binding to the LDL receptor, leading to increased plasma residence time (Nigon F et al., 1991) and an increased susceptibility to oxidation (Tribble DL et al., 1992).The reduction in large VLDL particles affected LDL particle size. Large VLDL particles are the precursors for the development of small-dense LDL particles (Krauss RM et al., 2004). MC or MD treatment to AD rats or HFD rats or HCD rabbits significantly decreased the serum VLDL level when compared to respective control group

Elevated plasma TG also affects HDL-C levels and HDL particle size. Through the actions of cholesterol ester transfer protein (CETP), intravascular exchange of neutral lipids and apolipoproteins occurs between TG-rich lipoproteins (TRL) and HDL. CETP activity is regulated by TG content (Fielding CJ & Fielding PE, 1995). Thus, elevated TG leads to the generation of TG-rich HDL particles, which are more susceptible to modification by hepatic lipase (HL) (Packard CJ, 2003). This modification leads to the formation of smaller HDL particles, which have a reduced plasma residence time, creating an environment of diminished reverse cholesterol transport (Xu Y 2003).

The reductions in plasma TG were accompanied by altered VLDL metabolism. VLDL is created through the combination of apo B and TG via the actions of microsomal transfer protein (Gibbons GF et al., 2004). MC or MD treatment to AD rats or HFD rats or HCD rabbits also significantly decreased the serum triglyceride level

Epidemiological studies have indicated that elevation in serum cholesterol constitutes a principle and independent risk factor in the etiology of atherosclerotic lesion and coronary heart disease. (Kanal WB et al., 1979; 1986) Hypercholesterolaemia is one of the important risk factors for coronary heart disease (CHD).

The plasma cholesterol level can generally be regulated by the absorption of dietary cholesterol, the excretion of cholesterol via fecal sterol or bile acid, the cholesterol biosynthesis, and the removal of cholesterol from circulation. As such, numerous previous studies have reported on the beneficial effects of HMG-CoA reductase and acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors on hypercholesterolemia and atherosclerosis.Statins competitively inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate. This conversion is an early rate-limiting step in cholesterol biosynthesis.

By inhibiting this enzyme, statins markedly reduce plasma concentrations of LDL and total cholesterol and to a lesser extent Apo-B and triglycerides and increase levels of HDL cholesterol. The mechanism of the LDL-lowering effect may involve both reduction of VLDL concentration and induction of the LDL receptor, leading to reduced production and/or increased catabolism of LDL. Lovastatin and simvastatin are inactive lactone prodrugs that are rapidly hydrolyzed to their active beta-hydroxyacid forms. The other statins are administered in their active forms. Saponin fractions of MC (175 mg/kg, p.o, /day/35days) in our study showed significant HMG CoA reductase inhibitory activity. HMG CoA reductase inhibitory activity was also seen in diabetic and insulin resistance models indicating that MC may exhibit antihyperlipidemic activity by inhibiting HMG CoA reductase enzyme.

Coronary heart diseases resulting from progressive atherosclerosis, remains the most common cause of death in our society. Atherosclerosis is primarily a lipid disorder affecting the arteries. Qualitative changes in lipoprotein structure and function, possibly occurring in the vicinity of or within the arterial wall, may enhance the atherogenic potency of the lipoproteins by altering their interactions with other cells and with the arterial wall itself. Atherosclerotic plaque contains foam cells that originate from macrophages saturated with cholesterol and also from smooth muscle cells. Accumulation of lipid in the arterial wall plays a crucial role in the genesis of atherosclerosis and has been the subject of extensive investigation since the time of Anitschkow Increased intracellular generation of reactive oxygen species has been proposed as a mechanism to tissue injury with a variety of pathological processes like ischaemia, inflammation, atherosclerosis and thrombosis (Mark et al. 1992). Lipids undergo peroxidative changes in the arterial wall, which eventually produce tissue injury. Increased free radicals can cause abnormal function of endothelial cells via reduced NO availability, and is believed to be an early event in atherogenesis (Ross, 1993). Diminished NO activity enhances the expression of transcription factor-nuclear kappa B (Barnes and Karin, 1997), which up-regulates the synthesis of inflammatory cytokines and adhesion molecules, leading to further complications and endothelial injury. In atherosclerosis inflammation may also alter the integrity of the vascular endothelium and cause exposure to thrombogenic material in plaque with clot formation and reduction in coronary flow (Airaghi et al. 1995).

Although the cholesterol-induced atherosclerotic lesions observed in rabbits do not resemble the typical atheromatous/fibrous plaques of human subjects, they

have some similarities to the human lesions, which include smooth muscle cellular proliferation, intracellular lipid inclusions, lipid depositions, etc.

Feeding rabbits with HCD in the present study thickened vascular wall of aortic musculature with fatty tissue, there was also formation of neointima containing vascular smooth muscle cells of tunica media with foam cells. Saponin fractions of MC&MD (175/55mg/kg p.o/day/35days respectively) treatment to HCD rabbits did not show formation of neointima containing vascular smooth muscle cells of tunica media and almost normal architecture. Hence MC & MD has significant antiatherosclerotic effect

Although several chemicals and drugs are generally used against atherosclerosis and related heart diseases, the indigenous drugs with a long descended heritage of traditional use are of supreme importance, to re-establish traditional claims with scientific interest. Clinical studies in India have consistently confirmed guggul (gum resin of *Commiphora mukul*) extracts improve lipid levels in humans (Malhotra et al. 1977). Guggulsterones are the active principles in the gum resin of *Commiphora mukul* (Dwivedi, 1996). Garlic *(Allium sativum)* is attributed to have antithrombotic, platelet antiaggregatory, hypolipidaemic, antimicrobial, diuretic and hypoglycaemic activities (Satyavati et al. 1976).The pharmacologically active constituents in *Allium sativum* are allyl sulphide, ajoene, allicin, etc (Dwivedi, 1996). Plumbagin, a known isolate from *Plumbago* species administered to hyperlipidaemic rabbits reduced serum cholesterol (Sharma et al. 1991). Feeding the extract of *Semecarpus anacardium* inhibited the progression of atherosclerotic lesion and promoted plaque regression and further helped in mobilization of lipids especially cholesterol from liver (Kurup et al. 1979). The active constituents of this plant are flavones and flavonoids (Arti et al. 1995). *M. oleifera* has been shown to contain β-sitosterol. The cholesterol lowering effect of *M. oleifera* may be due to the inhibition in reabsorption of cholesterol from endogenous sources in association with a simultaneous increase in its excretion into faeces in the form of neutral steroids(Komal Mehta et al., 2003)

Saponins are surface active substances of plant origin, which are poorly absorbed from the gastrointestinal tract (Basu & Rastogi, 1967; Birk, 1969). Some saponins have been shown to increase faecal steroids in man and in experimental animals, and in some instances to lower plasma cholesterol (Griminger & Fisher, 1958; Newman *et al.* 1958; Shibata, 1961;Topping, Storer *et al.* 1980), probably as a result of binding bile acids (Oakenfull & Fenwick, 1978). In rats faecal bile acid

excretion, especially primary bile acid excretion, and neutral sterol excretion increased with dietary soya-bean flour rich in saponins. Other saponins may have different effects on cholesterol metabolism. For instance those probably from *Saponaria oficinalis* partially reverse the hypercholesterolaemia caused by a high cholesterol diet in rats, and increase faecal excretion of bile acids and neutral sterols (Oakenfull *et al.* 1979). Alfalfa (lucerne) saponins in the diet prevent diet-induced hypercholesterolaemia in monkeys (Malinow, *et* al. 1977). Saponins from different plant species differ in structure (Basu & Rastogi, 1967; Birk, 1969), and may differ in their ability to bind sterols or in their biological effect when added to the diet. By virtue of their purported intestinal action, saponins could provide a non-systemic alternative to the commonly used HMG- CoA reductase inhibitors (statins). Because of their complementary mechanism of action, saponins could be particularly well suited for combination therapy with statins or other systemic hypolipidemic agents.

There are other mechanisms besides cholesterol complexation by which saponins are postulated to inhibit cholesterol absorption. Saponins form complexes with membrane cholesterol or extract cholesterol from membranes and this may explain the hemolytic activity of some saponins. *Gypsophylla* or *Saponaria* saponins decrease permeability and active glucose transport in isolated everted jejunal sacs (Johnson, I.T et al., 1986), with permeability changes persisting after saponin pretreatment. Saponins isolated from soy, *Saponaria officinalis*, or *Quillaia saponaria* altered the size or shape of micelles (Oakenfull, D. 1986), and this mechanism is a likely explanation for the inhibition of bile salt absorption by a variety of saponins (Oakenfull, DG & Sidhu, GS.1990). Plant sterols such as sitosterol and sitostanol, though structurally distinct from saponins, are thought to inhibit cholesterol absorption by competing with cholesterol for micellar solubility. Micellar exclusion of cholesterol was proposed as a potential mechanism for tiqueside (Harwood, H. J et al., 1993).In the present study HMG CoA reductase inhibitory activity of saponins of MC and MD is investigated and has found that saponins of MC has significant HMG CoA reductase inhibitory activity. Hence in addition to inhibiting cholesterol absorption from the GIT like any other saponins, Saponins of MC inhibit HMG CoA reductase enzyme.

Oxidative stress may play a role in the pathophysiology of diabetes and cardiovascular disease (Oberly LW 1988; Rao AV, 2002).

A multitude of *in vivo* studies have been performed utilizing antioxidants in experimental diabetic models. The effects of antioxidants on oxidative stress are measured through observable biomarkers like enzymatic activities of catalase, SOD, and GSH-reductase, as well as thiobarbituric acid reactants (TBARS) levels, an indirect measurement of free-radical production that has been shown to be consistently elevated in diabetes. Many animal studies have been completed with this aim in mind and indeed have shown that diabetes-induced alterations of oxidative stress indicators can be reversed when the animals are treated with various antioxidants.

Mekinova et al. (1995) demonstrated that supplementation of streptozotocin (STZ) diabetic rats with vitamins C, E, and beta-carotene for 8 weeks produced a significant reduction of TBARS levels, GHS, and GHS-Px, an increase in Cu-SOD, and no change in catalase activity in kidneys. Treatment with vitamins C and E was also shown to decrease urinary albumin excretion, glomerular basement membrane thickness, and kidney weight in STZ diabetic rats (Kedziora-kornatowska K et al., 2003). In the same study, vitamins C and E significantly lowered Malondialdehyde (TBARS) levels and GSH-Px activity while increasing catalase and SOD activities when compared to unsupplemented diabetic animals (Kedziora-kornatowska K et al., 2003). A study by Cinar et al.(2001) demonstrated that supplementation with vitamin E significantly lowered liver and lung TBARS levels and improved impaired endothelium-dependent vasorelaxation in STZ diabetic rat aorta. One study demonstrated that treatment of STZ diabetic rats with α-lipoic acid reverses SOD-induced vasorelaxation, potentially due to the elimination of excess superoxide/hydrogen peroxide and the recovery of basal NO (Rauscher F et al., 2001). Treatment with MC or MD significantly increased the SOD, Catalase and GSH levels and decreased LPO levels when compared to diabetic animals.

ROS can stimulate oxidation of low-density lipoprotein (LDL), and ox-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (Liu Y et al., 2002). As described earlier MC and MD has significant antiatherosclerotic effect in atherogenic diet rats and high cholesterol diet rabbits. Antioxidant activity of MC and MD can be one of the mechanisms for antiatherosclerotic effect. There is substantial evidence from in vitro studies that statins exert an antioxidant effect. Statins have also been shown to stimulate the activity of the antioxidant enzyme thioredoxin

(Haendeler J et al., 2001). Oxidation of LDL ex vivo has been shown to be inhibited by long-term statin therapy.

$^{\bullet}O_2^-$ can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC, all of which have been proven to be involved in micro- and macrovascular complications in the heart, which is an important target in diabetes and prone to diabetic cardiomyopathy leading to chronic heart failure, SOD and glutathione peroxidase expression as well as activity are decreased whereas catalase is increased in experimental models of diabetes (Maritim AC et al., 2003; Kaul N et al .,2003 Hayden MR et al., 2003).Study by Brands et al (2004). Investigated the effect of oxidative stress in the development of hypertension in diabetes using the SOD mimetic tempol in a Type 1 model of diabetes where NOS is pharmacologically inhibited with a NOS inhibitor, L-NAME. In this model, hyperglycemia causes hypertension implicating an important role for NO. Results of this study showed that if $^{\bullet}O_2^-$ is eliminated by tempol early in the disease process, the hypertension and decrease in glomerular filtration precipitated by diabetes are prevented. In the present study MC and MD have brought in regenerative changes changes with striations, branched appearance and continuity with the adjacent myofibrils in the cardiac sections of diaberic heart; regerative changes in the diabetic pancreatic cell islets and regerative changes in the diabetic kidney with minimal glomerular sclerosis and vacuolization. The antioxidant effect of MC and MD may have protected the organs from degeneration.

Few epidemiological studies have examined the association between serum antioxidants and cardiovascular disease or death (Riemersma RA. et al., 1991; Salonen JF et al., 1988; Salonen JT et al., 1985; Kok FJ et al., 1987; Kritchevsky SB et al., 1993). Singh et al., (1994, 1996) reported on plasma antioxidants, oxidative stress and the effect of antioxidant vitamins in acute myocardial infarction (AMI). There was a significant drop in vitamins C, E, A and ß-carotene, whereas lipid peroxides were significantly higher in AMI compared with controls [Singh et al., 1994]. Provision of vitamins A, C, E and ß-carotene in patients with AMI resulted in a decrease in serum lipid peroxides compared with a placebo group. Also, there were fewer complications in the treatment group [Singh et al., 1996].

Another prospective study was based on findings from Lipid Research Clinics Coronary Primary Prevention Trial.15 the level of total serum carotenoids had a protective association with coronary heart disease incidence. This was statistically significant among the placebo group All study participants had type IIA hyperlipoproteinemia.

Kumar and Das have suggested that an increase in free radical generation and a simultaneous decrease in the production of NO and antioxidant enzyme such as superoxide dismutase and vitamin E occurs in essential hypertension which is a well known risk factor for AMI.

Pinelli A. et al., (2004) observed that considerable ischemic alterations were observed in the animals treated with isoproterenol, including areas of myocardial necrosis, contraction band necrosis, increased plasma levels of cardiac necrosis markers (c-troponin I and myoglobin), and ele trocardiographic modifications (ST segment changes and T wave inversion). The myocardial infarction was attributed to the inotropic activity of isoproterenol leading to intracellular calcium overload. The cardiac necrosis phenomena appear to be associated with isoproterenol-induced lipid peroxide generation (as shown by the decrease in plasma Vitamin E levels) and increased procoagulant activity (a shortened PTT).

Oral pretreatment of MC or MD for 45 days significantly prevented myocardial alterations and restored the mitochondrial function at near normal state. These results confirm the efficacy of MC and MD in alleviating isoproterenol induced mitochondrial damage

CCl_4 metabolism by hepatocytes, and endogenous macrophage-like Kupffer cells (Badger, DA et al., 1996), results in severe hepatic necrosis and fibrosis. Liver injury in this established model derives from a mixture of free radicals and reactive oxygen species (Badger, DA et al., 1996, Sipes IG et al., 1991), lipid peroxidation (Terao et al 1984), activated Kupffer cells (Edwards MJ et al., 1993), and infiltrating PMN (Louis H.JL et al .,1998), each of which has an underlying role in the resulting liver damage.

Hepatoprotection by silymarin has been attributed to its antioxidant properties by scavenging prooxidant free radicals and increasing intracellular concentration of glutathione, a substance required for detoxicating reactions in liver cells. silymarin inhibits peroxidizing enzymes like lipoxygenase, thus blocking peroxidation of fatty

acids and damage to membrane lipids(Valenzuela A and Garrido A.1994; Rui YC 1991).MC and MD exhibited significant hapatoprotective activity and MD significantly increased the SOD, Catalase and GSH levels and decreased LPO levels when compared CCl_4 induced hapatotoxic rats. MC and MD may have hapatoprotective effect by the virtue of their antioxidant effects.

There is overwhelming epidemiologic evidence that insulin resistance and hyperinsulinemia are strongly associated with hypertension (Modan Met al., 1985) Similar to insulin resistance and hyperlipidemia, many published experiments have shown that high-fructose diets induce hypertension in animals, including rodents (Dai S & McNeill JH 1995; Erlich Y& Rosenthal T 1995; Suzuki M et al 1997; Verma S et al 1994). The mechanism of fructose-induced hypertension is not well understood, but such factors as also decreases uric acid production (Reiser S et al., 1985), hyperinsulinemia (Daly ME et al 1997), aldehyde formation (Vasdev S et al., 1998), and altered vascular reactivity (Verma S et al 1996) have been implicated. Fructose feeding induced hypertension in normal-fed and high salt–fed rats was associated with an increased expression of the angiotensin II type 1 receptor in adipose tissue (Giacchetti G et al 2000). Hyperinsulinism has also been associated with renal sodium retention (Silverberg AB et al., 1978). It has been recognized that insulin resistance may play a role in the development of essential hypertension in humans (Ferrannini E and DeFronzo RA 1991; Gans RO & Donker AJ 1991).

As sodium accumulation begins, compensatory mechanisms come into play, preventing further sodium or volume retention. A decrease in aldosterone has been reported in fructose-fed rat as well as in sucrose-fed humans (Hwang 1-S et al., 1989). Fructose-induced metabolic disturbances could play a role in sodium retention (Affarah HB et al., 1986). Alterations in sodium and calcium membrane transport may cause vascular hyper reactivity and increased vascular resistance. (Felicetta JV& Sowers JR 1988; Banskota NK et al., 1989). Hyperinsulinism has also been associated with renal sodium retention (DeFronzo RAet al., 1976) vascular smooth muscle hyper reactivity (Felicetta JV et al., 1989), and hypertrophy (Banskota NK et al., 1989)

In fructose-fed rats, the addition of clonidine to the drinking water prevents the development of hypertension but not of insulin resistance, hyperinsulinemia, and Hypertriglyceridemia (Hwang I-S et al., 1987); on the other hand, blockade of insulin secretion with octreotide prevents hypertension (Reaven GM et al., 1989). MC

significantly decreased the systolic blood pressure in FRD rats. MC may exert its antihypertensive effect by improving insulin sensitivity. It was observed in the present study that pressor response to Adr (1μg/kg, i.v), NA (1μg/kg, i.v) and PE (1μg/kg, i.v) significantly decreased after MC (10mg/kg, i.v) administration. Hence MC may prevent hypertension by modifying vascular reactivity and vascular smooth muscle hyper reactivity as described by Felicetta JV et al., (1989). MC also decreased uric acid production in isoprotereol-induced myocardial infarction in the present study and this may have significant implication on antihypertensive effect as described by Reiser S et al., (1985).

Chinese medicine Tang-Shen-Jiao-Nang (TSJN) may improve insulin resistance, lower the systolic blood pressure, and modulate muscle fiber composition in hypertensive and insulin-resistant fructose-fed rats (Li Y et al., 2001). Methanolic extracts of Bidens pilosa (Dimo T et al., 2002), Monascus purpureus (Hsieh PS & Tai YH.2003) Pterocarpus marsupium (Grover Jket al., 2005) and many more plants are studied for antihypertensive activity. MC can play a major role in the mechanisms underlying the pathogenesis of fructose-fed hypertension as demonstrated by the beneficial effects on blood pressure. MC may also exert its antihypertensive effects directly by reducing the increase of the total peripheral resistance induced by the high-fructose diet or by blockade of the calcium channels. These observations may be similar to the effect of nifedipine, which provokes its antihypertensive effects by reducing calcium-dependent vasoconstrictor tone of arterial smooth muscle (McLeay et al., 1983; Young et al., 1984, Masuda et al., 1990).

MC has shown a significant non competitive alpha adrenergic blocking effect on rat aortic strip and rat ancoccygeous muscle in our study.

Alpha-adrenergic receptor blocking agents, such as doxazosin, are effective antihypertensive drugs used in the management of essential hypertension. (JD. Neaton e al., 1993) A beneficial effect of doxazosin on insulin action has been suggested from several studies in subjects with essential hypertension, although these have been primarily uncontrolled studies with several using suboptimal means of assessing insulin action.(C. Giorda and M. Appendino 1993, P.-E. Andersson et al., 1994, S. Kageyama et al., 1993). Alpha-adrenergic blockers can be added to the antihypertensive regimen of insulin-resistant patients with confidence that insulin resistance will not be exacerbated. The use of doxazosin in subjects with hypertension and type 2 diabetes has been reported to improve glucose metabolism, despite some of

these studies also being limited by methodologic considerations.(C. Giordaet al., 1995, M. Giordano et al., 1995) Therefore, it is possible that in a more insulin-resistant population, a positive effect of doxazosin on insulin action. By the vertu of alpha adrenergic blocking effect MC may show beneficial in the management of hypertension and glycemic control.

Suzuki M et al (1992) have reported that Insulin sensitivity is better related than hyperinsulinemia to hypertension and that this insensitivity is partially reversible by alpha 1-blocker, bunazosin. Hence the insulin sensitizing effect of MC and MD may be related to alpha adrenergic blocking effect.

Cherksey et al. (1982) have reported that the adrenergic receptors on rat pancreatic islet cells are of the alpha 2-subtype.A. Kashiwagi et al (1986) report that New alpha 2-adrenergic blocker (DG-5128) improves insulin secretion. Ortiz-Alonso(1991) have reported that, MK-912, a potent new selective [alpha]2-adrenergic receptor antagonist was shown to decrease of fasting plasma glucose, increase of fasting plasma insulin, improve of β-cell function due to an increase in maximal B-cell secretory capacity; and increase in basal and-stimulated glucagon. Hence insulin secretion seen in the present study may also be due to the alpha adrenergic inhibitory effect of MC and MD.

M. Velasco et al (1985) in a study on hypertensive patients has reported that Prazosin appears to have a more beneficial effect on blood lipids and lipoproteins.

SW Rabkin (1993) has reported decrease in triglycerides and LDL cholesterol with increase in HDL cholesterol in patients receiving alpha adrenergic receptor blocker

In another study SW Rabkin et al., (1994) have reported significant favorable changes in HDL-C, total triglycerides, and VLDL-T between patients with mild to moderate hypertension and normal plasma lipids when treated with the alpha- blocker doxazosin compared with the beta-blocker atenolol. Erdem Akbay et al (2001) report that Terazosin an alpha blocker, gives a beneficial effect on the lipid profile in older symptomatic BPH patients with a higher ratio of dyslipidemia. Hence antihyperlipidemic effect of MC and MD may also be as a result of their alpha adrenergic blocking effect.

Many morphological, histological, physiological, and biochemical changes occur in the ovary during the estrous cycle. During the maturation of preovulatory follicles, ovulation takes place under the combined and balanced influence of ovarian and extra ovarian hormones. Imbalance in these hormones leads to irregularity in the ovarian

functions and duration of the estrous cycle (Prakash AO et al.,1979, Shivalingappa H et al., 2002, Circosta C et al., 2001) . The estrous cycle in the rats treated with MC and MD showed a decrease in the duration of estrous and the metestrous phases. It was also characterized by a prolongation of the proestrous phase. The prolongation of the proestrous phase indicates that maturation of the follicle in the preovulatory phase was delayed, leading to non-maturation of graffian follicle. Non-availability of matured graffian follicle was indicated by reduction in the estrous and the metastrous phases. Therefore, ovulation was inhibited. This result was further supported by histopathological studies in which the transverse section of the ovary showed the presence of primary or developing follicles. Ovary can be considered an aggregate of three endocrine tissues, the stroma, the follicle and the corpus luteum. The weights of these tissues constitute the net weight of the ovary. During the estrous cycle the weight of the ovarian tissue increases under the influence of gonadotrophic and steroidal hormones. The decrease in the weight of ovaries of the rats treated with MC or MD indicates a decrease in the activity of the stroma, the follicle, and the corpus luteum in the ovary. This decrease may be due to the non-availability of gonadotrophic or steroidal hormones or both (Shivalingappa H et al., 2002) . Atretic follicles are degenerating preovulatory follicles. The degeneration of preovulatory follicles takes place due to nonavailability of steroidal hormones (essential for their maturation and differentiation), non-availability of local estrogen produced by granulosa cells, or imbalance in endogenous steroid, protein and hormones. The presence of increased atretic follicles in the rats treated with MC or MD, indicates that the extract promotes the degeneration of preovulatory follicles. Cholesterol is the precursor for the steroidogenesis of ovarian endocrine tissues. The significant increase in ovarian cholesterol in the treated group indicates that cholesterol is not used for steroidogenesis. (Shivalingappa H et al., 2002) Abortion refers to the premature expulsion of the products of conception from the uterus. Abortion may be due to maternal exposure to chemicals, which can disrupt pregnancy and cause detachment of the embryo. (Feranada CG. et al., 2000).MC and MD at 500 mg/kg showed 100% abortifacient activity, while 250 mg/kg did not show abortifacient activity. However, it reduced the number of viable fetuses.

Inhibition of ovulation may be because of hormonal manipulation and may be the cause for the antifertility activity of MC or MD in the postcoital state of rats. Significant abortifacient potential may also be due to the antifertility activity. Hence,

it was investigated to know the antifertility activity of MC and MD and the involvement of estrogenic and progestrogenic mechanisms in the antiimplantation activity of MC and MD. MC and MD have shown highly significant antiimplantation activity when administered from days 1 to 7. The extracts were also given during different periods of gestation on successive stages of embryogenesis as described by Hafez(1970). From our results, it is evident that the extracts inhibited implantation when given at the zygotic stage (days 1-3) and the blastocyst stage (days 3-5).(Schmidt H 1995) Similar activity is reported in many plants.(Vohora SB et al., 1969) Many antifertility plant extracts are known to exhibit estrogenic activity in rats.(Gebrie E et al 2005) Estrogen causes an increase in protein synthesis, uterine weight, water uptake and retention of fluid leading to ballooning of the uterus.(Rifai Net al., 2001) In addition, estrogen also induces uterotrophic changes such as increase in diameter of the uterus, thickness of endometrium, height of endometrial epithelium, providing nonreceptive conditions for implantation.(Dhar SK 1995) Estrogen causes vaginal opening, which is a qualitative measure of estrogen potency. Presence of cornified cells in vaginal smears also indicates estrogen activity. Estrogen is known to increase uterine content of glucose, cholesterol, glycogen and alkaline phosphatase, thereby changing the uterine milieu and creating nonreceptive conditions in the uterus.(Rifai Net al., 2001) Administration of the MC or MD to immature rats at doses of 250 and 500 mg/kg did not show any change in uterine weight or histoarchitecture of uterus (uterotrophic changes) such as diameter of the uterus, thickness of endometrium or height of endometrial epithelium. There was no vaginal opening or presence of cornified cells in the vaginal smear. The treatment also did not show any change in the uterine content of glucose, cholesterol and alkaline phosphatase when compared with control group. Conjoint administration of ethanolic extract with ethinyl estradiol did not decrease the uterine weight and histoarchitecture of uterus (uterotrophic changes) when compared with ethinyl estradiol. MC or MD also did not cause any decrease in the uterine content of glucose, cholesterol and alkaline phosphatase. Hence, neither of the extracts have any estrogenic or antiestrogenic activities.

Pregnancy maintaince test and Clauberg's assay are commonly used in the bioassay for progestational activity.(Vogel HG 1997, Kuhnz Wet al., 1995, Tuba Z et al., 2000, McKim JMet al2001).Bilateral ovariectomy performed in rats during the first half of pregnancy results in termination of gestation, but if ovariectomy is performed

during the second half of pregnancy, abortion may not necessarily occur. This is due to the capacity of the placenta to produce progesterone and estrogen. It has been shown that pregnancy can be successfully maintained in rats ovariectomized during the first half of pregnancy by administration of sufficient quantities of exogenous progesterone alone or a combination of progesterone and estrogen. In the present study, unlike the administration of estrogen and standard progesterone, administration of estrogen and the ethanolic root extract to the ovariectomised pregnant rats did not maintain pregnancy.

Clauberg's assay is another bioassay of progestational and antiprogestational activities (Vogel HG1997). The histological changes in the uterus (*e.g.*, the endometrial proliferation) seen in estrogen-pretreated immature rabbits after the administration of progestational or antiprogestational compounds are assessed here in rabbits. Progestational activity of the compound was assessed by its ability to induce endometrial proliferation (increased uterine diameter and endometrial thickness) whereas its antiprogestational activity was assessed by the ability to inhibit endometrial proliferation induced by norethisterone, a progesterone analog. Administration of estrogen alone to the rabbits caused ramification of the uterus (increased endometrial epithelial cell height), but not proliferation. In the present study, administration of both estrogen and the ethanolic root extracts of at doses of 250 and 500 mg/kg showed ramification of the uterus but not proliferation, which was similar to the effect of estrogen and vehicle alone. Hence, MC and MD at doses of 250 and 500 mg/kg may not have progestational activity.

The negative effects of saponins on animal reproduction have long been known and have been ascribed to their abortifacient, antizygotic and anti-implantation properties (Tewary et al. 1973; Stolzenberg & Parkhurst, 1976). Saponins from broom weed (Gutierrezia sp.) and lechuguilla (Agave lecheguilla) or commercial pharmaceutical grade saponins caused abortion or death or both in rabbits, goats and cows when administered intravenously at concentrations above 2·3 mg/kg body weight (Dollahite et al.

1962). Saponins isolated from the crude extract of Gleditischia horrida, Costus speciosus Sm and Phytolacca dodecandra caused sterility in mice (Chou et al. 1971; Tewary et al. 1973; Stolzenberg & Parkhurst, 1976). Quin & Xu (1998) found that the butanol extract of Mussaenda pubescens was capable of terminating pregnancy in rats. Extracts of this plant are used as a contraceptive in the Fujian province of China. The

steroidal saponin isolated from Ornithogalum saundersiae injected into rats on the morning of pro-oestrous at a level of 9mg/kg inhibited oestrogen production and prolonged the period of dioestrous (Tamura et al. 1997). Saponins were found to be extremely strong stimulators of luteinising hormone release from cultured hypophysial cells (El Izzi et al. 1989; Benie et al. 1990) but their action was neutralised in the presence of serum indicating a passive membrane-permeabilising effect (El Izzi et al.1992).

MC or MD did not show any effect on the Epididymal sperm density, Sperm motility, Serum Cholesterol, ALP activity, Testosterone level, Body weight and weights of Testes, Epididymis, seminal vesicles and prostate glands after 60 days of treatment male rats. And hence they do not have any effct on the male reproductive system. Saponins have been shown to have both positive and negative effects on the viability of human sperm cells in vitro with some ginseng saponins increasing motility as well as progression of sperm (Chen et al. 1998) while Sesbania sesban saponins were spermicidal at 1·0–1·3 mg/ml (Dorsaz et al. 1988).